Hydroxyiminodisuccinic acid (HIDS): A novel biodegradable chelating ligand for the increase of iron bioavailability and arsenic phytoextraction

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Hydroxyiminodisuccinic acid (HIDS): A Novel Biodegradable Chelating Ligand for the Increase of Iron Bioavailability and Arsenic Phytoextraction

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Abstract

The influence of biodegradable chelating ligands on arsenic and iron uptake by hydroponically grown rice seedlings (*Oryza sativa* L.) was investigated. Even though the growth solution contained sufficient Fe, the growth of rice seedlings gradually decreased up to 76% with the increase of pH of the solution from 7 to 11. Iron forms insoluble ferric hydroxide complexes at neutral or alkaline pH in oxic condition. Chelating ligands produce soluble 'Fe-ligand complex' which assist Fe uptake in plants. The biodegradable chelating ligand hydroxyiminodisuccinic acid (HIDS) was more efficient then those of ethylenediaminetetraacetic acid (EDTA), ethylenediaminedisuccinic acid (EDDS), and iminodisuccinic acid (IDS) in the increase of Fe uptake and growth of rice seedling. A total of 79±20, 87±6, 116±15, and 63±18 mg dry biomass of rice seedlings were produced with the addition of 0.5 mM of EDDS, EDTA, HIDS, and IDS in the nutrient solution, respectively. The Fe concentrations in rice tissues were 117±15, 82±8, 167±25, and 118±22 μmol g^{-1} dry weights when 0.25 mM of EDDS, EDTA, HIDS, and IDS were added to the nutrient solution, respectively. Most of the Fe accumulated in rice tissues was stored in roots after the addition of chelating ligands in the solution. The results indicate that the HIDS would be a potential alternative to environmentally persistent EDTA for the increase of Fe uptake and plant growth. The HIDS also increased As uptake in rice root though its translocation from root to shoot was not augmented. This study reports HIDS for the first time as a promising chelating ligand for the enhancement of Fe bioavailability and As phytoextraction.

Keywords: Arsenic, Iron, Chelating ligands, Rice (*Oryza sativa* L.), Hydroponics, Bioavailable, HIDS.

1. Introduction

Iron is an essential micronutrient for plants, which plays important roles in respiration, photosynthesis, and many other cellular functions such as DNA synthesis, nitrogen fixation, and hormone production (Vert et al., 2002). Although abundant in nature it forms insoluble ferric hydroxide complexes (also known as Fe-plaque) at neutral or alkaline pH in oxic condition (Guerinot and Yi, 1994). The Fe-plaque formation in the rhizosphere soils, however, results in the Fe deficiency to plants. In nature, rhizospheric microbes exude siderophores to the root-plaque interface. These siderophores solubilize ferric iron in the rhizosphere, render its bioavailability, and plants uptake the Fe by specific membrane receptors (Romheld, 1987).

Elevated levels of As in soil from natural and anthropogenic sources is a threat to plants' health (Rahman et al., 2008). Remediation of contaminated soil is important to prevent As deposition in food crops and its subsequent transfer into the human body through the food chains (Rahman et al., 2008). Phytoremediation becomes a promising alternative and environmentally safe technology for the remediation of environmental pollutants (Raskin et al., 1997; Tu et al., 2002). An essential prerequisite for phytoremediation of contaminated soil is solubility and bioavailability of As (Fitz and Wenzel, 2002). But the solubility and bioavailability of As becomes reduced by adsorption to variable charged minerals (Fe and Al) at alkaline pH (Xu et al., 2008). In the past decade, chelant-enhanced phytoremediation has received much attention (Pastor et al., 2007). This technique aims to cleanse polluted soils by solubilizing the toxic metals, allowing it to be accumulated in plants that would subsequently remove toxic metal from the site. Publications on chelant-enhanced phytoremediation have increased steadily to about 15-20 per year in the last few years, indicating that this is a growing and active research field (Nowack et al., 2006).

Research on the interaction of plants with chelating ligands started in the 1950s with a view to reduce the deficiencies of the essential nutrients such as Fe, Mn, Cu, and Zn (Wenger et al., 2005). Among all soil-applied Fe fertilizers, synthetic Fe(III)-chelates, mainly Fe(III)-chelates of polyaminecarboxylic acids with phenolic groups, such as ethylendiamine di(*o*-hydroxyphenylacetic) acid (EDDHA), and ethylendiamine di(2-hydroxy-4-methylphenylacetic) acid, are the most effective and commonly used (Alvarez-Fernandez et al., 2005). On the other hand reports on As phytoextraction by chelating ligands is limited though a number of investigations have been conducted on chelant-enhanced phytoextraction of Pb, Zn, Hg, Cu and some other heavy metals (Luo et al., 2005). Ethylenediaminetetraacetic acid (EDTA) has been very popular to achieve this purpose, but it is quite persistent in the environment because of its low biodegradability. This, in combination with its high affinity for heavy metal complexation, results in an increased risk of leaching. EDTA also impairs plant growth severely, even at low concentrations (Bucheli-Witschel and Egli, 2001).

Biodegradable chelating ligands, such as ethylenediaminedisuccinic acid (EDDS), Hydroxyiminodisuccinic acid (HIDS), and iminodisuccinic acid (IDS) would be good choice and alternative to less biodegradable EDTA. The physicochemical properties of EDDS, EDTA, and IDS have already been discussed and tested for the phytoextraction of heavy metals by a number of researchers (Helena et al., 2003; Evangelou et al., 2007). HIDS is a new chelating ligand introduced by Nippon Shokubai Co. Ltd. It is one of the highly biodegradable (biodegradation rate is about 22.4% within 48 h) and safe chelating ligands. It traps and inactivates various kinds of metals ions over a wide rage of pH, particularly Fe^{3+} and Cu^{2+}, as well as Ca^{2+} and Mg^{2+}; shows high stability in harsh conditions and high temperature (80 ºC); is highly soluble in aqueous alkaline solution (Sokubai, 2009). Because of high degradation rate and high stability constant with Fe^{3+} (pKaFe^{3+} is 12.5) of HIDS, we become interested to investigate the effectiveness of the chelating ligand for the increase of Fe bioavailability and phytoremediation of As. The EDTA, EDDS, and IDS were also used in the present study to compare the results of HIDS. Our research approach was to find a biodegradable and eco-friendly chelating ligand that is more desirable then EDTA or EDDS for Fe bioavailability and As phytoextraction.

2. Materials and Methods

2.1. Seed sterilization

Rice seeds of BRRI dhan 29 were collected from Bangladesh Rice Research Institute. The seeds were surface-sterilized before using them in the experiment. For sterilization, about 100 g seeds were soaked in 200 mL of 1% methyl-1-butylcarbamoyl-2-benzimidazole carbonate solution for 10 min. After that, the seeds were washed by deionized (DI) water (using an E-pure system (Barnstead)) and kept in DI water at 20 ºC for 24 h. The seeds were then washed and transferred to DI water of 45 ºC for 2 min, and of 52 ºC for 10 min.

2.2. Chemicals

Stock solutions of EDTA, EDDS, HIDS, and IDS were prepared by dissolving ethylenediamine-N,N,N',N'-tetraacetic acid (Dojindo Molecular Technologies, Japan), ethylenediamine-N, N'-disuccinic acid (Chelest), tetrasodium 3-hydroxy-2,2’-iminodisuccinate (Nippon Syokubai, Japan), and tetrasodium iminodisuccinate (Bayer) in 0.1 M sodium hydroxide, respectively. Other reagents were of analytical grade or better. All solutions were prepared with DI water.

2.3. Nutrient solution

Sterilized rice seeds were germinated on pre-sterilized bloating paper (seed bed) with standard murashige and skoog (MS)(Murashige and Skoog, 1962). Iron concentration in the experimental solution was 0.36 mM while its concentration was 27.8 mg L^{-1} in pre-experimental solution (used for growing rice seedling prior to the experiment). The pH of the pre-experimental solution was adjusted to 6.5 while the pH of experimental solution was 9.0. Rice seedlings were grown on the seed bed for 1 wk. In preparing MS culture solution, $FeSO_4 \cdot 7H_2O$ was used as Fe source instead of NaFe(III)-EDTA.

2.4. Experimental setup

Rice seedlings were transferred to the experimental solution after one week of growth in pre-experimental solution. In the experimental solution, rice seedlings were grown in two steps. In the first step, rice seedlings were grown with different concentrations of chelating ligands (up to 2.50 mM) to observe the effect of chelating ligands on Fe uptake. In the second step, 6.0 µM of As ($Na_2HAsO_4 \cdot 7H_2O$) was added to the nutrient solutions containing 1.0 mM of chelating ligands to see the effect of chelating ligands on Fe and As uptake. Iron concentration in the experimental solution was 0.36 mM, and the pH of the solution was adjusted to 9 using 0.1 M KOH. About 100 mL of the solution was taken into 250-mL polystylene bottles with three replications, and three uniform seedlings were cultivated in each bottle. The experiment was performed following randomized design. Rice plants were grown in a plant growth chamber and the conditions in the chamber were set as 14:10 h light/dark schedule, 100-125 µ E m^{-2} s^{-1} light intensity, 22(±2) ºC temperatures. Rice seedlings were grown in experimental solution for 5 d.

2.5. CBE-extraction of Fe-plaques

At harvest, the shoots were cut from 1 cm above the roots and separated. The Fe-plaques from root surfaces were extracted using citrate-bicarbonate-ethylenediaminetetraacetate (CBE)-technique, a modified method of dithionite-citrate-bicarbonate extraction by Taylor and Crowder (1983) to determine the real amount of Fe and As contents in rice tissues. The CBE solution was prepared from 0.03, 0.125 and 0.050 M of sodium citrate, sodium bicarbonate, and EDTA, respectively. Roots were treated with 30 mL of CBE solution for 60 min at room temperature. The roots were then rinsed with deionized water for 3 times, and the rinsed water was added to the CBE-extracts to make a total of 30 mL.

2.6. Sample preparation

After rinsing with deionized water for four times, the root samples were kept on clean absorbent paper to remove the water from the root surfaces. Both the root and the shoot samples were dried at 65 ºC until they reached in a constant weight. Then the dried samples were weighted and taken into 50-mL polyethylene tubes for digestion. Five mL of 65% HNO_3 were added to the sample and kept for 12 h. The samples were heated on a heating block at 95 ºC for 2 h. After cooling to room temperature, 3 mL of 30% hydrogen peroxide were added, and the samples were heated again at 105 ºC for 20 min. Then, the digests were diluted to 30 mL with DI and analyzed for As and Fe.

2.7. Chemical analysis

Arsenic and Fe were analyzed using graphite-furnace atomic absorption spectrometer (Z-8100, Hitachi, Japan). Certified standard reference material 1573a (tomato leaf from NIST, USA) was used to check the accuracy of analysis. Arsenic concentration in certified standard reference materials was 0.112±0.004 µg g^{-1} dry weight (all the reported data in this article are expressed as dry weight) while the measured concentration was 0.114±0.002 µg g^{-1}. The concentrations detected in all samples were above the instrumental limits of detection (≥ 0.01 µM in water sample).

All chemical reagents used in this experiment were of analytical grade. Glassware and dishes were washed with detergent and 1 N HCL solution, and rinsed with DI water for eight times before use. In each analytical batch, at least two reagent blanks and three replicate samples were included.

3. Results and Discussions

3.1. Effect of pH on rice growth

Rice seedlings were grown in nutrient solution adjusted to different pH ranging between 6 and 11. Results show that the biomass production of rice seedlings was affected by the pH significantly. The highest biomass of rice seedling (83±7 mg) was observed at pH 7, which was about 16, 19, 43, and 76% higher than those at pH 8, 9, 10, and 11, respectively (Fig. 1). The rice growth remain unchanged, and even died at pH 10 and 11. Rice plants have a tendency of higher Fe uptake than that of other plants (Becker and Asch, 2005). But the pH of the growth medium plays an important role in Fe bioavailability and uptake. Even though the Fe is sufficient in growth medium, it forms insoluble ferric hydroxide complexes at alkaline pH in oxic condition (Cohen et al., 1998). Therefore, Fe bioavailability and uptake decreases drastically. In the present study, it was observed that the Fe concentrations in tissues of rice seedlings were highest at pH 7 compared to those at other pHs (Fig. 2). This trend of Fe uptake in rice tissues is correlated to that of biomass production of rice seedlings (Fig. 1). The result implies that the influence of pH on rice growth is the ultimate effect of reduced Fe bioavailability and uptake. Moreover, Fe concentrations on root surfaces of rice seedlings were lowest at pH 7 and 8 compared to those at other pHs (Fig. 2). High level of Fe on root surfaces of rice seedling at pH 11 reveals the formation of Fe-hydroxides (Fe-plaque) on root surfaces, which decreased the Fe uptake in rice tissues. Formation of Fe-plaques on the roots of wetland plants (Hansel et al., 2001) and hydroponically grown rice seedling (Hu et al., 2005) have also been reported. The precipitation of ferric (oxyhydro)-oxides (FeO_x) and its association with phytoplankton surfaces, both in natural conditions and laboratory cultures, has been reported by Tang and Morel (2006). Robinson et al. (2006) also found the occurrence of Fe-plaque on aquatic macrophytes collected from the Taupo Volcanic zone, New Zealand.

The Fe deficiency results in Fe-chlorosis in green leaves, which retards plant growth, and leads to the reduction of crop yields (Guerinot and Yi, 1994). The results of the present study also reveal that the growth of rice seedling decreased drastically at higher pH, which is the consequence of Fe-chlorosis.

3.2. Influence of chelating ligands on Fe uptake-translocation

Influence of EDDS, EDTA, HIDS, and IDS on Fe uptake and translocation in rice seedlings were investigated at different concentrations of the ligands ranging between 0.1 and 2.5 mM. Results showed that Fe uptake in rice seedling differed significantly with the type and concentrations of the chelating ligands. Iron uptake was highest at 0.25 mM of the chelating ligands compared to the control treatment. Iron uptake decreased gradually with the increase of chelating ligand concentrations above 0.25 mM (Fig. 3). The effectiveness of HIDS and EDDS in the increase of Fe uptake in rice tissues was higher than that of EDTA and IDS. Iron concentrations in roots of rice seedling were 35±3 and 44±2 μmol g^{-1} when the HIDS concentrations in the nutrient solution were 0.10 and 0.25 mM, respectively. These concentrations were significantly higher than those of other chelating ligands.

Iron concentrations in shoots of rice seedlings were significantly lower than those in roots, and were about identical up to 0.25 mM of chelating ligand treatment. Iron content in shoots decreased with the gradual increase of chelating ligands from 0.25 to 2.50 mM (Fig. 3). The results indicate that the translocation of Fe from roots to shoots was not affected by lower dose of the chelating ligands. The translocation of Fe was inhibited by the chelating ligands at higher doses (> 0.25 mM).

Although abundant in nature, Fe is often unavailable to plants, especially at neutral or alkaline pH, because of the formation of insoluble ferric hydroxide complexes in oxic condition (Robinson et al., 2006). Precipitation of Fe in the rhizosphere, however, may result in the Fe deficiency to the plants. Chelating ligands are used in agriculture as additives in micronutrient fertilizers for the increase of Fe bioavailability. Although some chelating ligands have been reported to increase Fe uptake/translocation in plant, inhibition of Fe uptake/translocation by ligands has also been reported. Chaney et al. (1972) reported that bathophenanthrolinedisulfonate (BPDS) was the most effective inhibitor of Fe uptake/translocation, followed by EDTA > DTPA (diethylenetriaminepentaacetic acid) > CDTA (diaminocyclohexanetetraacetic acid) >> EDDHA. The BPDS inhibited ^{59}Fe movement to the exudate by 99.7% even at the lowest level of competitor. The BPDS inhibits Fe translocation by 10-100 times compared to those of EDTA, DTPA, or CDTA. Chaney et al. (1972) also observed that EDDHA, the chelator with the highest Fe^{3+} stability constant, only slightly inhibited or actually promoted Fe uptake/translocation, whereas the BPDS with the highest Fe^{2+} stability constant was a severe inhibitor. Thus, stability constant of Fe-ligand ($\log K_{FeL}$) would be one of the important determinants for the promotion or inhibition of Fe uptake/translocation.

3.3. Effect of chelating ligands on rice growth

Rice seedlings were grown in alkaline nutrient solution (pH 9) containing 0.10, 0.25, 0.50, 1.00, and 2.50 mM of chelating ligands and 0.36 mM of Fe. Results show that the growth of rice seedlings was increased with the increase of HIDS and EDTA concentrations up to 1.0 mM, and the growth was decreased at 2.5 mM of chelating ligand concentrations (Fig. 4). The highest biomass production (141±21 mg) of rice was observed when 1.0 mM of EDTA was added to the nutrient solution followed by 127±8, 82±19, and 75±4 mg for HIDS, EDDS, and IDS, respectively.

Chelating ligands have been used to enhance Fe bioavailability (Alvarez-Fernandez et al., 2005). The concentration of chelating ligands in the nutrient medium is important for the solubilization of precipitated Fe and the increase of its bioavailability. In the present study, it was observed that the rice seedling produce highest biomass at 1.0 mM chelating ligand concentrations, and the growth remain unchanged, and even died at higher concentration (>1.0 mM).

Although the growth of all organisms is dependent on the acquisition of the proper quantities of trace elements, excess amount of some metals such as Fe, zinc, manganese, and copper produce toxic effects (Morel and Hering, 1993). However, ferric ions and their complexes, which have low solubility in aquatic system, are extensively buffered by chelation (Morel and Hering, 1993), and increase their dissolved concentration. The dissolved concentration of Fe determines its rate of uptake by the organisms. Anderson and Morel (1982) reported that the Fe uptake rate in laboratory cultures of the marine diatom *Thalassosira weissflogii* is a unique function of the free ferric ion concentration at the presence of 10^{-5} M of various chelating ligands (1.4×10^{7} cells L^{-1}). Hudson and Morel (1990) reported that in Fe-limited culture of marine diatom *Thalassosira weissflogii* (10^{7} cells L^{-1}) containing 10^{-8} M Fe and 10^{-5} M EDTA and with white-light illumination, both the thermal dissociation of FeEDTA and its photoreduction and reoxidation contribute to the formation of the dissolved inorganic Fe(III) pool responsible for the Fe uptake. In this case, growth of rice seedlings was inhibited by the free ferric ion that was increased by the addition of higher level of chelating ligands.

Toxicity of chelating ligands on plants has not been studied extensively. So, it is difficult to interpret the direct toxicity of chelating ligands on plants. Since most of the chelating ligands are synthetic compounds, no nutrient carriers in the plasma membrane are thought to exist (Berne and Levy, 1998). Also, synthetic chelates cannot slip through the plasma membrane as they are too large and polar to move through the plasma lemma lipid bilayer (Berne and Levy, 1998). Tanton and Crowdy (1972) observed that most solutes moved into some endodermal passage cells adjacent to the casparian strip intracellularly to the other side of the strip, and then extracellularly to the xylem. The passage cells may include the aquaporins and there may be selectivity toward molecules. Paul et al. (2003) reported that Swiss chard uptakes a considerable amount of EDTA from chelator-buffered hydroponic solution through transpirational flow that occurs via apoplastically.

3.4. Influence of chelating ligands and As on rice growth

Chelating ligand treated rice seedlings were grown with and without As to investigate the effect of As and chelating ligands on rice growth. Results show that As does not have a consistent effect on rice growth as chelating ligand has. Rice growth was not affected by As when chelating ligand was not treated. The highest growth of rice seedling was observed in HIDS treated medium. The inconsistent effect of chelating ligand and As on rice growth suggest that in the presence of chelating ligands lower level of As in the growth medium does not affect rice growth significantly. It has been reported that rice growth is not affected by low level of As though the growth decrease drastically with the increase of As in the soil. Abedin and Meharg (2002) also reported that low level of As in water (about 2.0 mg L^{-1}) does not show toxicity to both rice germination and rice growth, but the rice germination and growth were aversely affected by higher As level.

3.5. Influence of chelating ligands and As on Fe uptake/translocation

Iron uptake in rice seedling was affected by chelating ligands and As significantly. Iron concentration was measured both in root surfaces and plant tissues. Results show that the Fe concentration was higher in rice root surfaces of control treatment (without chelating ligands) while its concentration was higher in plant tissues of ligand treated nutrient solution (Fig. 5). The highest Fe contents were found in tissues of rice seedlings treated with EDTA or HIDS and As. Increasing Fe uptake by chelating ligands, especially EDTA and HIDS, can be explained by the adsorption of As(III)-EDTA/-HIDS complex on the Fe-plaques of rice root surfaces and dissociation of the complex to release of Fe(III)-EDTA/-HIDS into solution. The release of Fe(III)-EDTA/-HIDS into the culture solution results in the increase of Fe uptake. Adsorption of metal-EDTA to the surface of Fe oxides and dissociation of the complex and release of Fe(III)-EDTA has been reported by Nowack and Sigg (1997).

Strong ligands, such as EDTA, complex with metals in natural systems. Adsorption of uncomplexed EDTA on metal oxides (Fe-oxides, Al-oxides) has been studied previously (Bowers and Huang, 1985; Blesa et al., 2000). The EDTA has been reported to exist as complex species of metals (mainly CaEDTA, ZnEDTA, and Fe(III)EDTA) in natural waters (Xue et al., 1995). Dissolution reactions of Fe-oxides in the presence of metal-EDTA complexes have also been reported by Nowack and Sigg (1997).

3.6. Arsenic uptake/translocation affected by chelating ligands

Arsenic contents in roots, shoots, and root surfaces of rice seedling were determined to asses the effect of chelating ligands on As uptake. Results show that As was stored mostly in roots followed by shoots and root surfaces (Fig. 6). Previous studies with rice also reported higher content of As in rice roots (Abedin et al., 2002). The higher storage of As in roots and lower translocation to shoots can be explained by the reduction of arsenate to arsenite in roots, complexation with thiols, and sequestration in the root vacuoles (Zhao et al., 2009).

Formation of Fe-plaque on rice root surfaces and its effect on As uptake in rice have been well explained in literature (Liu et al., 2006). Although Fe-plaque inhibits the As uptake (Zhang et al., 1998), increase of the uptake of toxic and nutrient elements in plants and organisms by Fe-plaque has also been reported (Ye et al., 2001). The effects of Fe-plaque on the uptake of nutrient and/or toxic elements depend on the amount of Fe-plaque on root surfaces (Zhang et al., 1998). Otte et al. (1989) reported higher concentration of Zn in roots of *Aster tripolium* L. coated with 500-2000 nmol Fe cm^{-2} compared to those coated with less than 500 or more than 2000 nmol Fe cm^{-2}. Even though the increasing amount of Fe-plaque elevates As accumulation on the root surfaces, it does not affect As uptake in rice shoots. The Fe-plaque acts as "buffer" to prevent the translocation of As from roots to shoots Liu et al. (2004).

Present study also report that the As contents in roots and shoots were higher in rice seedlings grown with chelating ligands compared to those grown without chelating ligands (Fig. 6). Arsenic content in roots was highest when the rice seedlings were grown with HIDS while the content was identical when grown with EDTA, EDDS, or IDS. The results suggest that chelating ligands increased As uptake in rice root significantly, though its translocation form root to shoot was not increased. The use of chelating ligands, especially the EDTA, EDDS, IDS, etc. for the increase of heavy metals have been studied extensively (Jean et al., 2008; Marques et al., 2008). Present study reports a better/comparable performance of HIDS to that of others studied previously for the first time.

Arsenate has a high adsorptive affinity to Fe oxides (Zhao et al., 2009). Chelating ligands solubilization/desorption As from the Fe-plaque of rice roots, and rice plant readily uptakes desorbed/soluble As from the nutrient solution. The results of the present study reveal that the HIDS is stronger then EDTA, EDDS, or IDS for dissolution/desorption of precipitated As. Since the EDTA is not readily biodegradable, and is persistent in the environment, the biodegradable HIDS would be a good alternative to EDTA in the phytoextraction/phytoremediation of As.

4. Conclusions

The use of chelating ligands in the phytoextraction of toxic metals and in the increase of essential nutrient elements is not new at all. Especially, the EDTA and EDDS have been widely used in agriculture for long time to serve the above purposes. The use of EDTA, however, has the disadvantage that it is quite persistent in the environment due to its low biodegradability. Therefore, looking for biodegradable chelating ligands is an important concern to the researchers. In this study the effectiveness of HIDS for the increase of Fe bioavailability and As phytoextraction was investigated, and the results were compared with those of EDTA, EDDS, and IDS. The Fe limiting condition was induced by increasing the pH of the growth solution. Results show that the performance of HIDS was more effective then that of other chelating ligands. HIDS is a newly introduced, biodegradable and environmentally harmonious chelating ligands with high chelating capability. Therefore, it would be a good alternative to the EDTA.

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Figure Captions:

Fig. 1: Growth of rice (*Oryza sativa* L.) affected by the pH of nutrient solution. Results are presented as mean and the error bars express ± SD (n = 3).

Fig. 2: Fe concentration in roots, root surfaces, and shoots of rice seedling (*Oryza sativa* L.) as affected by the pH of nutrient solution. Results are presented as mean and the error bars express ± SD (n = 3).

Fig. 3: Fe uptake and translocation in rice seedling (*Oryza sativa* L.) as affected by chelating ligand concentrations in the nutrient solution. Results are presented as mean and the error bars express ± SD (n = 3).

Fig. 4: Growth of rice seedling (*Oryza sativa* L.) affected by chelating ligand concentrations in the nutrient solution. Results are presented as mean and the error bars express ± SD (n = 3).

Fig. 5: Fe concentration in root surfaces and plant tissues (roots and shoots) of rice seedling (*Oryza sativa* L.) as affected by chelating ligands and arsenic in the nutrient solution. As(+) and As(-) indicate with and without arsenic, respectively.

Fig. 6: As concentration in roots, shoots, and root surfaces of rice seedling (*Oryza sativa* L.) as affected by chelating ligands in the nutrient solution.

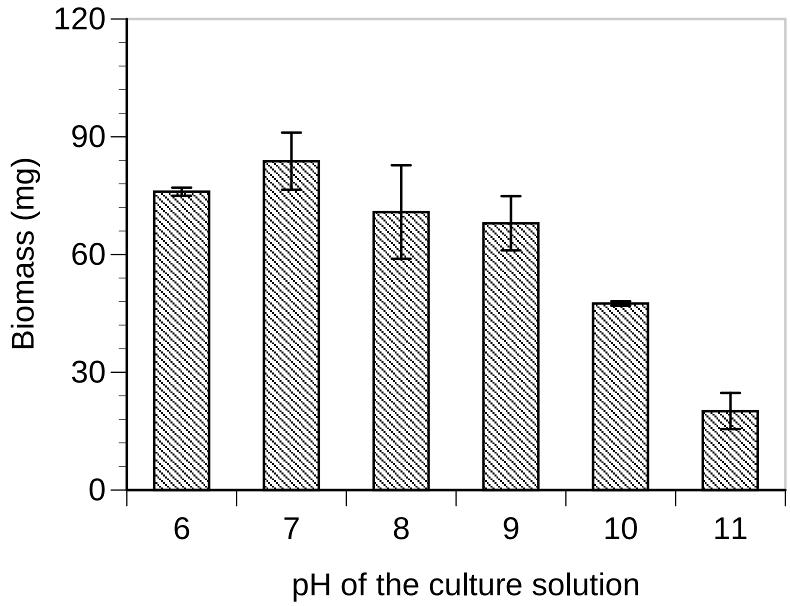

Fig. 1: Growth of rice (*Oryza sativa* L.) affected by the pH of nutrient solution. Results are presented as mean and the error bars express ± SD (n = 3).

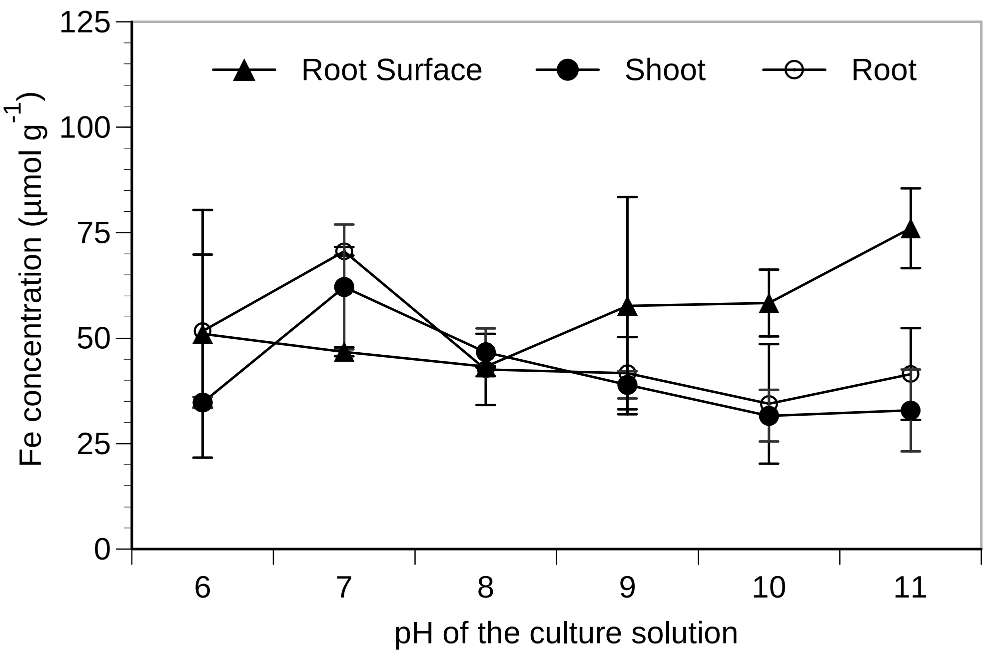

Fig. 2: Fe concentration in roots, root surfaces, and shoots of rice seedling (*Oryza sativa* L.) as affected by the pH of nutrient solution. Results are presented as mean and the error bars express ± SD (n = 3).

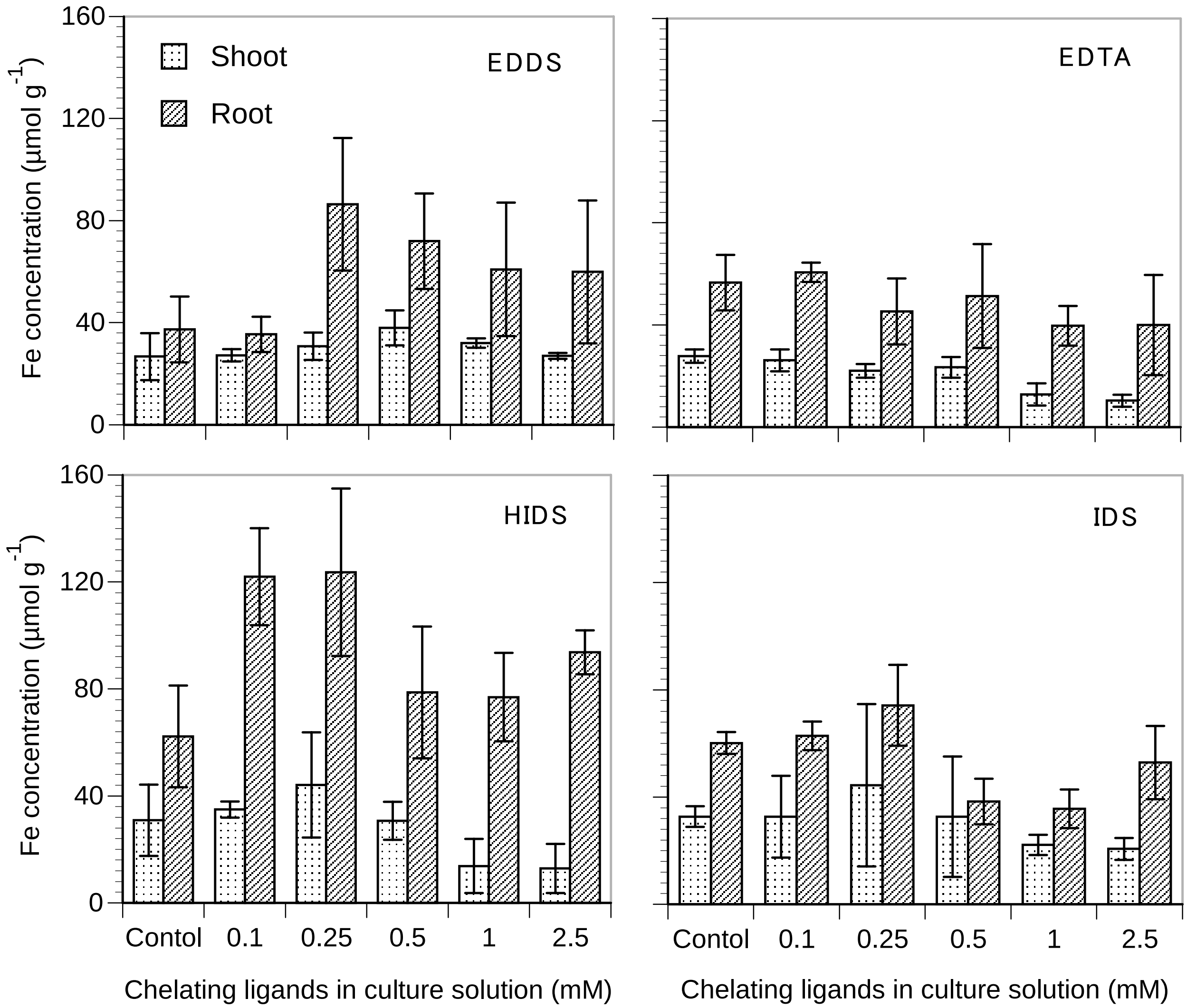

Fig. 3: Fe uptake and translocation in rice seedling (*Oryza sativa* L.) as affected by chelating ligand concentrations in the nutrient solution. Results are presented as mean and the error bars express ± SD (*n* = 3).

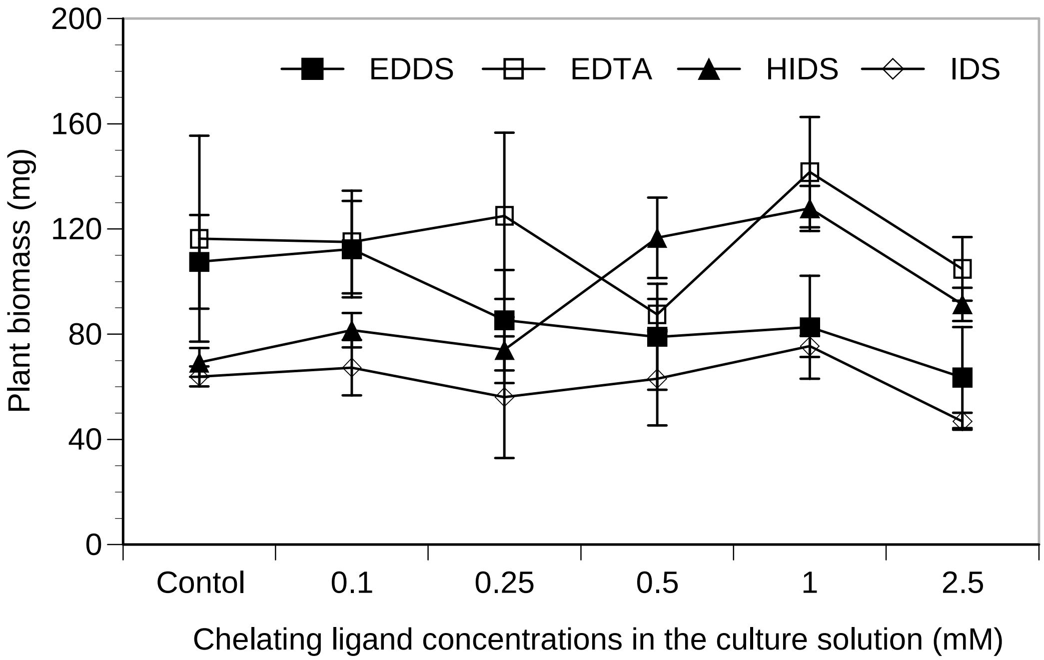

Fig. 4: Growth of rice seedling (*Oryza sativa* L.) affected by chelating ligand concentrations in the nutrient solution. Results are presented as mean and the error bars express ± SD (n = 3).

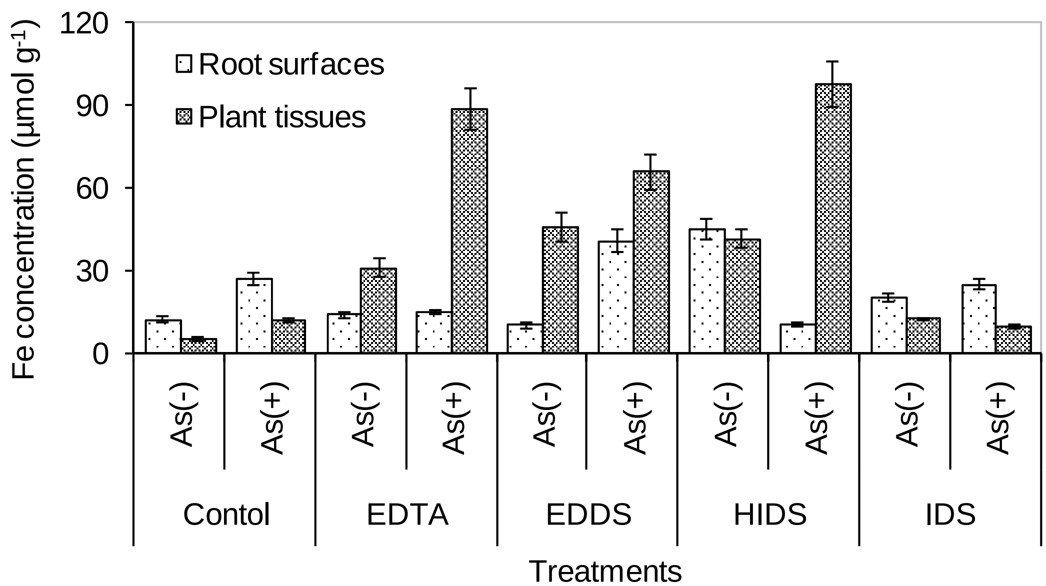

Fig. 5: Fe concentration in root surfaces and plant tissues (roots and shoots) of rice seedling (*Oryza sativa* L.) as affected by chelating ligands and arsenic in the nutrient solution. As(+) and As(-) indicate with and without arsenic, respectively.

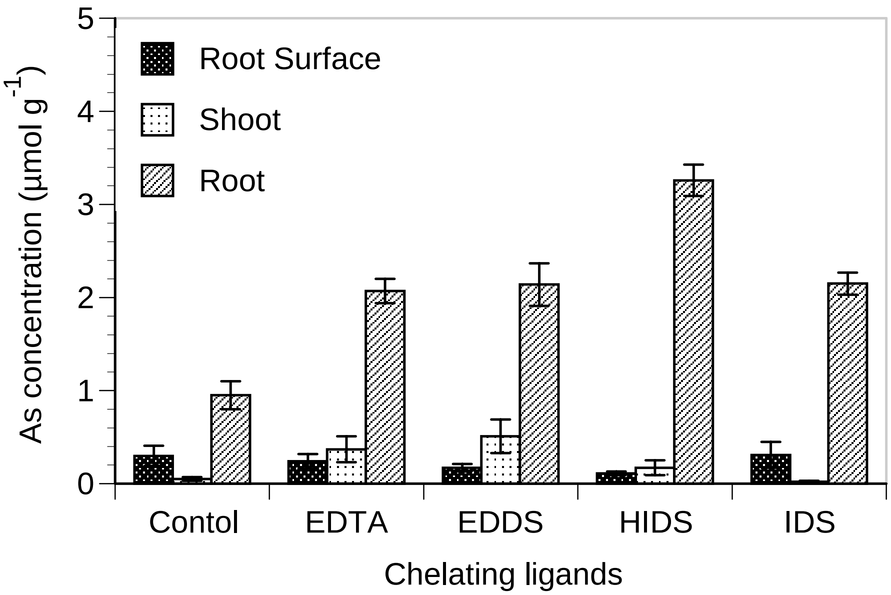

Fig. 6: As concentration in roots, shoots, and root surfaces of rice seedling (*Oryza sativa* L.) as affected by chelating ligands in the nutrient solution.